Review Article

APPLICATION OF NANO COMPOSITES IN CALVARIA HEALING AND BONE DEFECTS: A LITERATURE OF REVIEW

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Abstract

Bone grafting has numerous applications in dentistry such as alveolar ridge augmentation during implant therapy and reconstructive surgeries involving large bone defects. Bone regeneration is a multifactorial phenomenon which contributed to several factors. Fresh autogenous cancellous bone grafts are currently the "gold standard" treatment for bone regrowth. They are the most effective agents in the rapid bone graft healing, without triggering an immune response. They also provide maximum compatibility with the host tissue. Several researches were done to generate materials to promote bone regeneration procedure. However, due to the unavailability of autogenous bone and the problems associated with surgery, non-autogenous, replacement material remains a treatment option. Clinical research has shown the combination of bone grafts with other treatments can increase the engraftment, formation of bone tissue and bone defect healing. Being osteoconductive, osteoinductive and osteogenisis recommended for bone graft materials. Application of the biological agents as mediators of bone regeneration stills the safe method. Clinical research has shown the combination of bone grafts with other treatments can increase the speed of engraftment and formation of bone tissue, as well as improve the healing of bone defects. A suitable alternative bone graft treatment should be osteoconductive, osteoinductive, and osteogenic. Thus, the purpose of this study was to determine the crown discoloration following regenerative endodontic treatment using the PubMed and Medline database English literature by the terms "Nano composites", "Calvaria healing" and "Calvaria healing". In conclusion based on the reports Nano composites + autograft have positive effects on bone defects and calvaria healing.

Key words: Bone defects, Calvaria healing, Nano composites.

Introduction

Bone grafting has numerous applications in dentistry such as alveolar ridge augmentation during implant therapy and reconstructive surgeries involving large bone defects. Bone tissue regeneration is one of the most challenging aspects of tissue engineering with a great impact on the lives of millions of people worldwide.[1] Although autogenous bone grafts constitute the 'gold-standard' of bone defect reconstruction, their inherent donor-related limitations and resorption issues in large defects render their clinical use difficult and sometimes ineffective.[2] Bone tissue engineering emerges as the treatment of choice in bone reconstitution by exploiting different combinations of the basic bioengineering tools: stem cells, scaffolds and growth factors. Bone defects happen because of the periodontal disease, tumor resection, skeletal deficiency/disorder, abnormal development and trauma.[3] Numerous approaches have been undertaken to treat bone defects with the goal of regenerating the lost osseous tissue thereby regaining function. Available treatments to treat bone defects sometimes have limitations supply and transplant rejection/incompatibility along with surgical side effects such as infection, disease transmission or neurovascular injury.[4] These limitations led to tissue engineering approaches for repairing skeletal defects. Bone grafts have been used to augment osseous defects in the dental and orthopedic fields.[5] Application of the biological agents as mediators of bone regeneration stills the safe method.[6] Autogenous bone grafts are known as gold standard and preferred augmentation material.[7] Autogenous bone needs donor site morbidity, prolonged operation times and high costs.[7] Autogenous bones are effective agents in rapid bone graft healing, without triggering an immune response and highest compatibility with the host tissue.[8] Clinical research has shown the combination of bone grafts with other treatments can increase the engraftment, formation of bone tissue and bone defect healing. Being osteoconductive, osteoinductive and osteogenisis recommended for bone graft materials.[9] Scaffolds, growth factors and osteoblasts are tissue engineering elements have been used to enhance bone regeneration.[10]

The guided bone regeneration technique, described using a barrier membrane to promote bone augmentation by application of a barrier membrane. Guided bone regeneration has been widely used to encourage the formation of new bone in osseous defects by restricting the infiltration of soft tissues.[11] Advantages introduced for this method such as no need to second surgical procedure to remove absorbable membrane.[12] Clinical research has shown the combination of bone grafts with other treatments can increase the speed of engraftment and formation of bone tissue, as well as improve the healing of bone defects.[13] A suitable alternative bone graft treatment should be osteoconductive, osteoinductive, and osteogenic.[14] Nanocomposites contains high level of the hydroxyapatite and collagen and gained much attention as bone grafts not only due to their composition and structural similarity with natural bone but also because of their unique functional properties such as larger surface area and superior mechanical strength than their single phase constituents.[15] Because of complexity of the osteoclast and osteoblasts and the other factors made the clinicians to search for alternative bone graft substitutes in regenerating process in human.[16]

Material and Methods

The keywords used for the literature search for this review was peer-reviewed articles following coronal discoloration × Nano composites × Calvaria healing × Bone defects. Among them, the papers were fit the criteria selected and available full-text articles read. Related articles were also scrutinized. Hand search was also driven. The search was carried out using Biological Abstracts, Chemical Abstracts, and the data bank of the PubMed and Medline database updated to 2017. The references found in the search were then studied in detail.

Guided bone regeneration

Many techniques introduced for regenerating damaged periodontal tissues.[3] Guided tissue regeneration (GTR) therapy was introduced to achieve a repopulation of the periodontal ligament fibroblasts.[17] Also, guided bone regeneration (GBR) is the best documented for the treatment of localized bone defects.[18] Advantages introduced for this methods such as no need to second surgical procedure to remove absorbable membrane.[12] The clinical and economic effects of bone defect treatments are staggering.[11] Several researches were done to produce biological materials for GBR and regrowth. Because of the unavailability of autogenous bone and the problems associated with surgery, non-autogenous, replacement material remains a treatment option.[10]

Nanocomposite

It is suggested combination of bone grafts with bioactive materials improve the engraftment, bone formation and bone defect healing.[13] Application of the biological agents as mediators of GBR is a safe technique.[19] Many different groups have tried to manipulate the mechanical properties (e.g., stiffness, strength, and toughness) of scaffolds through the design of nanostructures (e.g., the inclusion of nanoparticles or nanofiber reinforcements in polymer matrices) to mimic bone's natural nanocomposite architecture.[20] To better mimic the nanostructure in natural extracellular matrix, over the past decade, scaffolds manufactured from nanofibers, nanotubes, nanoparticles, and hydrogel have recently emerged as promising candidates in producing scaffolds that resemble the extracellular matrix and efficiently replace defective tissues. Because natural tissues or organs are nanometer in dimension and cells directly interact with nanostructured extracellular matrix, the biomimetic features and excellent physiochemical properties of nanomaterials play a key role in stimulating cell growth as well as guiding tissue regeneration.[21] One tissue itself represents a biological nanocomposite composed of organic (collagen) and inorganic components, with a hierarchical structure ranging from the microscale to the nanoscale. Nano biomaterials and nanocomposites represent promising platforms in bone tissue engineering with a capacity to recapitulate the organization of natural extracellular matrix and the generation of functional bone tissues through osteomimetic architecture. The inherent properties of nanocomposites, such as increased wettability, roughness, and surface area, can also promote biomaterial-driven bone regeneration through increased protein adsorption, nutrient exchange, and porosity relative to macroscale biomaterials.[22] Nanocomposite scaffolds provide structural support for the cells, while changes to the nanoscale level of tissue hierarchy may have significant effects on cell-scaffold adhesion, integrin-triggered signaling pathways and cellular function; indeed, nanoscale features have been shown to have regulatory effects over multiple aspects of osteoblast and bone derived stem-cell behavior including adhesion, migration, proliferation, cell signaling, genetic expression, and stem cell fate.[22] Consequentially, biomaterial design has focused on the introduction of nanoscale elements that elicit directed cellular behavior while imparting structural and mechanical advantages to the bone construct to induce the formation of functional tissues. Current methodologies employed in the fabrication of nanocomposites include electrospinning and molecular self-assembly.[23]

Scaffolds

For the bone tissue engineering, a scaffold should be osteoconductive and highly porous to facilitate bone formation. Different classes of materials have been utilized for scaffold fabrication including a variety of ceramics and polymers. Calcium phosphate-based ceramics are popular scaffolding materials because their chemical structures are similar to the mineral phase of bone. A number of natural and synthetic polymers are also currently being employed in bone tissue engineering, especially in the repair of non-weight bearing site, such as cranium. More and more cellular and animal studies reported that chitosan-based scaffolds exhibited.[24]

Bisphosphonates

Bisphosphonates are phosphonate back bone molecules with different side chains. At least two groups of the bisphosphonates identified, including non-amino-bisphosphonates such as etidronate, elodronate and tiludronate with no nitrogen atoms and the amino-bisphosphonates like risedronate and alendronate, which have a nitrogen atom in their structure.[9] Bisphosphonates are pyrophosphate analogues with affinity for the hydroxyapatite.[6] Based on previous literature, bisphosphonates, non-hydrolysable analogues of inorganic pyrophosphate are the most prominent pharmaceutical drug for bone turnover.[25] Bone remodelling includes a balanced process of bone resorption through osteoclasts, increase in the mineral density of bone and bone formation by osteoblasts.[8] The bisphosphonates prevent bone resorption via the osteoclast to internalize the bisphosphonate and inducing its apoptosis.[7] Bisphosphonates has other biological effects such as joint cells, macrophages or chondrocytes. Risedronate improves cartilage lesions on the joints in guinea pig models. Numerous investigations were done to produce biological materials for bone regeneration and regrowth. Based on the unavailability of autogenous bone and the problems associated with surgery, non-autogenous, replacement material remains a treatment option.[10] It is reported combination of bone grafts with bioactive materials improve the engraftment, bone formation and bone defect healing.[13]

Risedronate

Risedronate acid is one of the important bisphosphonates and has demonstrated frequent beneficial properties on osteoarthritis progression. Risedronate decrease bone mineral loss at ligament attaches to bone and improves periarticular bone and ligament mechanical properties in rabbit.[19] In a study on effects of Risedronate on mandibular bone density, bone structure and bone metabolism in established glucocorticoid-induced osteoporosis in growing rats, Fujita *et al*[26] reported Risedronate improved prednisolone-induced decreases in trabecular bone. Risedronate also increased bone density and bone mineral content in trabecular bone. Co-application of the Risedronate and non-steroidal anti-inflammatory drug therapy in the early stages of osteoarthritis preserves trabecular bone mass and bone marrow lesion in rat.[7] Hoseinzadeh *et al*[27] studied effect of nano-capsules containing risedronate on calvarial bone formation in rabbit: radiography and biochemical investigation after 12 weeks bone formation in nano risedronate + autograft and autograft were 0.31 ± 0.03 and 0.25 ± 0.02, respectively, while in nano risedronate and control groups were 0.11 ± 0.01 and 0.08 ± 0.02, respectively. Significant differences were observed in bone density in nano risedronate + autograft as 0.37 ± 0.01, while the density in the autograft, nano risedronate, and control groups were as 0.32 ± 0.01, 0.14 ± 0.01, and 0.09 ± 0.02, respectively. In all stages of the study, the nano risedronate + autograft group had better bone formation in comparison to the other groups. Different filling materials of defects had no effect on blood hematology indexes). These results suggest nano risedronate + autograft has positive effects on calvarial bone defects healing in rabbit.[27]

Chitosan

Chitosan is a naturally biodegradable and bioactive biomaterial polymer which derived from the shells of crustaceans.[28] Chitosan is a biocompatible natural biopolymer that is a copolymer of N-acetyl- glucosamine and N-glucosamine units. It is acquired from chitin by depolymerization and partial deacetylation. Chitosan aids play a key role in immune system such as activation of macrophages and cytokine stimulation. It is reported chitosan promotes collagen synthesis and angiogenesis in the wound healing and tissue remodelling phases of wound repair.[28] There is growing interest on application of the organic-inorganic hybrid materials such as chitosan-based composite membranes.[4] Chitosan has a hydrophilic surface which promotes proliferation and mineralization of bone-forming cells.[29] It is reported chitosan-based bioelectret membrane to improve the bioactivity of the GBR membrane.[30] Due to its various biological characteristics, chitosan has been widely investigated as a bone substitution material and a membrane material in orthopedic and periodontal applications. Three-dimensional matrices of chitosan were also designed and tested for cell attachment as tissue engineering scaffolds. It is reported incorporation of copper into chitosan scaffolds promotes bone regeneration in rat calvarial defects.[3]

Hydroxyapatite

Hydroxyapatite has also attracted a great deal of attention recently.[13] Hydroxyapatite has been widely used in medicine because it is osteoconductive and has excellent biological affinity with bony tissue, possessing a similar chemical composition and structure as the mineral phase of bones. As a result, Hydroxyapatite is accepted as a bioactive scaffold material for guided bone regeneration. It has been reported that when Hydroxyapatite is combined with chitosan for bone tissue engineering, it can increase the bioactivity and mechanical properties of the materials.[13] Biological factors such as growth factors and cells are also typically required to effectively repair challenging bone defects. Bone morphogenetic protein-2 (BMP-2) has been shown to be a promising therapeutic agent promoting bone regeneration when delivered locally, but it has been demonstrated that adenovirus mediated BMP gene therapy can lead to harmful side effects such as tumorigenesis.[10] To provide specific and optimal biological activity, it is essential to design an appropriate carrier that retains BMP-2 and releases it slowly for bone formation. Several materials have already been evaluated as BMP carriers, such as collagen and other inorganic materials.[12] Although these materials can induce bone formation at orthotropic sites, they still have disadvantages such as the potential risk of immunogenicity, fragility, etc.[2] Therefore, we have focused on alginate for BMP delivery. Alginate is a natural anionic polysaccharide that is already approved by the FDA for human use as a wound dressing.[2] It has been widely used in cell culture and drug delivery, and its uses in long-term culture of osteocytes have been extensively documented. It has been reported that alginate and chitosan molecules form a polyelectrolyte complex through ionic interactions, and Ca^{+2} crosslinking reactions were also found in alginate/ Hydroxyapatite scaffolds as a result of divalent cations. Bone reconstruction in rat calvarial defects by chitosan/hydroxyapatite nanoparticles scaffold loaded with unrestricted somatic stem cells and revealed bone regeneration on the scaffolds, also the amounts of regenerated new bone for unrestricted somatic stem cells scaffold was significantly greater than the scaffold without cell and untreated controls. Therefore, the combination of scaffold especially with unrestricted somatic stem cells is considered as a useful method for bone regeneration.[15]

Eggshell

Avian egg shell has high level of mineral composition that can be used to produce hydroxyapatite, the major inorganic part in bone repair. The composition of avian eggshells differs slightly among species but is mainly a mineral matrix composed of calcium carbonate (97.4%), magnesium phosphate (1.9%) and triphosphate (0.7%).[8] Protein distribution differs based on the structures of the egg. Protein-polysaccharides are the main consists of the eggshell matrix and mainly containing 11% polysaccharides and 70 % proteins.[16] Chondroitin sulphates A and B were also composed 35% of the egg shell total polysaccharides. It is reported application of the hen eggshell had safe and inexpensive in rabbit bone defect model. In a study on effects of ostrich eggshell combined with eggshell membranes in healing of cranial defects in rabbits. Reported application of the ostrich eggshell powder had no effect on bone regeneration in rabbits. Large ostrich grafts are suitable as onlay graft, however a complementary osteosynthesis is suggested to improve osteointegration.[16]

Bioglass

There are two major groups of glass-based osteogenic scaffolds: glass-ceramic and glass-polymer porous composites. It has been demonstrated that silicon found in glass enhances angiogenesis as well as gene expression regulating osteogenesis and growth factor production in osteoblasts. Several studies have confirmed that silicate-based scaffolds are capable of stimulating osteogenesis.[30] Accordingly, silicon has been successfully incorporated into bioceramics in order to augment bioactivity and osteostimulatory effects.[10] Osteogenic and angiogenic growth factors have previously been utilized in bone tissue engineering, but the prospect of a single scaffold capable of inducing both osteogenesis and angiogenesis without exogenous growth factors has exciting implications.[12] Silicate bioglass as well as some ceramic scaffolds have been shown to posses this dualinductive attribute.[30]

Polymers

Natural and synthetic polymers are often used as scaffold materials for bone tissue engineering because of a well-balanced combination of properties, including biodegradability, biocompatibility, porosity, and ease of handling.[20] Naturally-derived materials, such as collagen and fibrin proteins, or chitin-derived chitosan polysaccharide, are also an option for bone tissue engineering.[21] Such materials may confer greater cell adhesion and functional support properties than synthetic materials, but in most cases, this is offset by several disadvantages. Natural polymers often offer less control over mechanical properties, sometimes exhibit immunogenicity, and frequently exist in finite supply; therefore, they are difficult and expensive to obtain. Osteoconductive biomaterials for scaffold construction Allogenic bone derivative Demineralized bone matrix, Ceramics Hydroxyapatite, Tricalcium phosphate, Biphasic calcium phosphate, Calcium carbonate Polymers Poly (lactic acid), Poly (glycolic acid), Poly (lactic-co-glycolic acid), Poly (propylene fumarate), Polycaprolactone, Polyamide Chitosan. Metals Titanium Magnesium Alloy Zinc, Bioglass Silicon Calcium-silicate, Thermoresponsive N-isopropylacrylamide, Poly (polyethylene glycol citrate-co- N-isopropylacrylamide), however, do not suffer from these shortcomings and have been a more important source of biomaterials for osteoconductive scaffold construction. Synthetic polymers can be produced on a large scale using reproducible and tunable methods, providing fine control over mechanical and physical properties. They have a well-documented history of clinical application in craniofacial bone reconstruction, especially in children. Synthetic polymers like poly (lactic acid) (PLA), poly (- glycolic acid) (PGA), and various iterations of combined poly(lactic-co-glycolic acid) (PLGA) have been used for a range of clinical applications, including critical-sized craniofacial defect repair.[8] It is reported the amount of new bones was significantly increased in the group of nanohydroxyapatite/chitosan/poly (lactide-coglycolide) scaffolds seeded with human umbilical cord mesenchymal stem cells. On the basis of previous studies in vitro and in subcutaneous implantation of the nude mice, the results revealed that the nano hydroxyapatite and chitosan also enhanced the bone regeneration by nano-hydroxyapatite/chitosan/ poly (lactide-coglycolide) scaffolds seeded with human umbilical cord mesenchymal stem cells in the calvarial defects of the nude mice at early stage.[30] In conclusion based on the reports Nano composites +autograft have positive effects on bone defects and calvaria healing.

Effects on bone regeneration

Mostly, the complicated and multiple fractures due to trauma or age (mostly at hip joint, that is, femur head fractures) are supported with prosthetic implants for proper healing. These implants are comprised of various materials known as biomaterials. Nevertheless, after 10–15 years on average, the traditional implant failure is associated with biomaterial associated inflammation, loosening, wear or tears debris, osteolysis and autoimmune reactions.[9] These snags urge for the development of biomaterials with greater cytocompatibility and long-lasting life, with higher patient's quality of life. The role of nanotechnology and nanomaterials therefore becomes very pivotal. Various nanocomposites, materials and particles have been applied to mimic the growth of bone tissues, lower the autoimmune reactions and keep check on microbial infections.[14] Herein, we also mainly focus on the nanomaterials role in bone tissue repair, support, and maintenance. Organic bone tissue has various protein (collagen, fibronectin, laminin, and vitronectin) and water as soft hydrogel nanocomposites, whereas hydroxyapatite and $Ca_{10}(PO_4)_6(OH)_2$ are hard inorganic components for the bone. The hydroxyapatite is present in nanocrystal line form which is 20–80 nm long and 2–5 nm thick, whereas the other proteins in the extracellular matrix are also at nanoscale size. This structural analogy allows the nanomaterials to interact easily with bone tissue and influence its functionality. Among the proposed nano-scaffolds for bone regeneration, Cerium (Ce-HA) based structures are among the leading candidates for bone tissue engineering. Similarly, a Mg-hydroxyapatite/collagen type I scaffold may also have great utility in bone regeneration.[15] Materials, at nanoscale, have been reported with better cell functionality than micro or macro scaled materials. The extracellular matrix provides scaffolds for the growth, proliferation and influence functionality of various cells. The nanoscale materials mimic the intrinsic and extrinsic pathways of osteocyte differentiation and mobility. Cells in various parts of the body exist in either two-dimensional (2D) or 3D environment, for example, stem cells in the intestinal crypts exists in 2D environment, whereas stem cells in bone marrow exist in 3D environment. The nanomaterials may provide the desired environment for the proliferation of various cells in a bone niche. Similarly, the magnetic nanoparticles in addition to influence on osteocytes intrinsic pathways, may also act as mechanical stimulus that will help in the healing process. The silk fibroin-hydroxybutyl chitosan blended nanofibers successfully provided scaffold for the growth of porcine iliac endothelial cells. The nanofibers provided typical extracellular matrix to cells, where these cells formed endothelial monolayer with higher confluency.[30] Bioactivity is the ability for a material to mimic response in living system. The orthopedic bioactive materials should elicit the biological response at interface and build a strong bond between the material and bone tissue. Hence, the role of bioactivity is inevitable for biomedical applications of biomaterials. The bioactive materials for bone repair are mainly divided into osteoconductive and osteoproductive, depending upon the rate of implant and its tissue interaction. The bioactive materials are mainly fabricated by either tailoring of bioactive composites and coatings or molecular surface tailoring. The later one is ideal for bone growth promoting factors, that is, BMPs. They are considered most important factors for the proliferation and growth of the bone tissue. The nanoarrays of gold has immobilizing effect on BMP-2, which allows the controlled release of BMP-2 that may have important role during the bone tissue repair via osteoblast.[10] The nanostructure arrays of various biomaterials (for example, polymethyl methacrylate 120 nm pit and 100 nm diameter size with 300 nm interspace) have been reported with efficient osteogenic differentiation of bone marrow mesenchymal stem cells BMSCs.[15] The materials not only promoted cell attachment, proliferation, spreading and osteogenic differentiation of adipose derived stem cells, but also enhanced the expression of angiogenic factors. The combination of the hydroxyapatite scaffolds with nano-surface and ASCs could enhance both osteogenesis and angiogenesis in a rat critical-sized calvarial defect model.

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